I1-030

7-28-97

CALFED Bay-Delta Program office
1416 Ninth Street, Suite 1155
Sacramento, California 95814

re. CALFED RFP, 1997 Category III, Ecosystem Restoration Projects and Programs

Dear Sir,

I am submitting two "Inquiry Submittals" for review. The proposed projects are in response to the CALFED RFP, 1997 Category III, Ecosystem Restoration Projects and Programs. I believe that both projects fit with the criteria outlined in the RFP and intend on submitting full proposals to CALFED during your next funding cycle. I would appreciate any comments you have with respect to improving or focusing the scope of each proposal.

Sincerely,

Mark S. Okihiro

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I. Executive Summary:

Project Title: **Developmental Effects and Biomarker Expression in Early Life Stages of Four Native Fish Species (chinook salmon, rainbow trout, delta smelt, and white sturgeon) as Ecological Indicators of Pesticide and Metal Exposure**

Principle Investigators: Mark S. Okihiro, DVM, Ph.D. (PI)
David E. Hinton, Ph.D. (Co-PI)
Swee J. Teh, Ph.D. (Co-PI)

Project Description and Primary Biological/Ecological Objectives: The primary goal of the proposed study is to assess the impact of pesticide and metal contaminants on early life stages (ELS) of four fish species native to the Sacramento River and Delta: chinook salmon (*Oncorhynchus tshawytcha*), Delta smelt (*Hypomesus transpacificus*), rainbow trout (*Oncorhynchus mykiss*), and white sturgeon (*Acipenser transmontanus*). While not on the priority list, rainbow trout and white sturgeon were selected to serve as surrogates for steelhead trout and green sturgeon respectively. ELS (embryos and larvae) were chosen because of their greater contaminant sensitivity, and a biomarker approach selected in order to expand characterization of the contaminant response. To fully assess contaminant impact on ELS, we propose using gross pathology, histopathology, and three biochemical assays: metallothionein, acetylcholinesterase (AChE), and ethoxyresorufin-O-deethylase (EROD). Biomarkers were selected for their utility, sensitivity, and specificity. It is anticipated that the proposed suite of markers will allow for the development of specific "contaminant response profiles" resulting in linkage of groups of bioeffects in ELS with specific contaminant classes. In so doing, we expect to accurately define tolerance limits for specific contaminants when exposed to ELS of native fish species. Specific objectives include: 1) to identify which ELS have the highest sensitivity to specific metal and pesticide contaminants; 2) to identify target organs, developmental defects, histologic lesions, and biochemical alterations associated with specific metals and pesticides; 3) to compare contaminant response based on species exposed, ELS exposed, and contaminant class; 4) to establish detailed "contaminant response profiles" for ELS of native fish species based on data gathered from laboratory exposures and to determine if "response profiles" can be used to classify contaminant laden field samples; 5) to compare the biomarker approach using ELS of native species with conventional EPA triple-species tests; and 6) to begin to establish an ELS contaminant response data base which can be used to set specific water quality criteria relevant to native fish species.

Approach/Tasks/Schedule: In phase I (yr 1), ELS of the 4 native species (each species designated as a task) will be exposed under laboratory conditions to metal (copper and zinc with salmonids; selenium and cadmium with smelt and sturgeon) and pesticide (diazinon and molinate) contaminants. Larval exposures will be conducted from 0-7 days post-hatch. Embryonic exposures will also be 7 days, but number of exposures will vary based on length of embryonic development. In phase II (yr 2), water samples will be collected from 6 field sites on the Sacramento River and Delta. Tasks 5-8 in phase II are again designated by species. Selection of field sites will be based on current and historic contaminant and bioassay data, geographic location, and rainfall. Collection of field water samples will be coordinated with the UCD Aquatic Toxicology Laboratory which has an ongoing study to perform EPA triple-species assays on water samples from the Sacramento River and Delta. ELS of each species with the highest sensitivity (identified in phase I) will be exposed to field water

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samples. The same suite of biomarkers will be assayed for in both phases. Phase II results will be compared with results from Phase I, as well as with results from EPA triple-species assays.

Justification: Although there has been extensive study of the Sacramento River and Delta, little information is available with respect to contaminant effects on native fish species. Water quality criteria exist, but are based primarily on conventional EPA triple-species tests and there is no way of knowing whether bioassay results utilizing fathead minnows (*Pimephales promelas*) are relevant to native species. In addition, conventional bioassays (relying on lethality) provide minimal data with respect to sublethal effects and contaminant specificity. The biomarkers proposed by this study will address both of these deficiencies. Studies utilizing histopathology reveal that diazinon exposure to embryonic medaka is associated with retinal necrosis, and that molinate induces CNS hemorrhage in larval striped bass. Metallothionein induction is specific for exposure to metal contaminants and AChE inhibition correlates with organophosphate exposure. While it does not provide specific information with regard to latent mortality, a biomarker approach utilizing a 7 day ELS exposure can readily be substituted for conventional assays using fathead minnows, and will greatly improve the sensitivity and specificity of the assay protocol. Finally, the development of "contaminant response profile" data base can be expanded as new contaminants enter the system and will allow regulatory agencies to set water quality criteria based on known effects to ELS of native fish species.

Budget Costs: Tasks in phase I involve laboratory exposures and cost are dependent on number of ELS used: task 1) chinook salmon, 6 ELS ($180,461); task 2) rainbow trout, 4 ELS ($143,264); task 3) Delta smelt, 3 ELS ($116,146); and task 4) white sturgeon, 2 ELS ($91,491). Phase I tasks involve field validation with only one ELS assay and charges vary with expenses involved with acquiring eggs: task 5) chinook salmon ($102,527); task 6) rainbow trout ($102,527); task 7) Delta smelt ($104,507); and task 8) white sturgeon ($104,507).

Applicant Qualifications: Dr. Okihiro is a veterinarian with 20 years of laboratory and field research experience, and extensive training in the fields of pathology, fish embryology, histology, and carcinogenesis. Dr. Okihiro has performed histopathological analyses of larval striped bass exposed to molinate and thiobencarb, and has assisted in studies involving wild striped bass larvae and evaluation of retinal lesions induced by diazinon exposure to medaka embryos. Dr. Teh has a Ph.D in the field of comparative pathology and extensive experience with enzyme- and immunohistochemistry, fish pathology, carcinogenesis, and morphometry. Dr. Teh has conducted preliminary experiments exposing ELS of delta smelt to diazinon. Dr. Hinton is a tenured UCD faculty member with 28 years of experience in the fields of aquatic toxicology and carcinogenesis. Dr. Hinton has overseen development of an AChE inhibition assay for use with ELS of medaka and will provide guidance with respect to experimental design and interpretation of data.

Data Evaluation: Prevalence and degree of biomarker response will be correlated with species, ELS, contaminant type and concentration. The benefits and utility of "contaminant response profiles" generated in phase I will be further evaluated in year 2 when assays are conducted using water samples collected from field sites. Results from field validation biomarker assays will also be compared with EPA triple-species tests conducted on duplicate samples.

Coordination with other Programs/Compatibility with CALFED objectives: Both phases of the study will be coordinated with Dr. Doroshov's (UCD) efforts to culture and rear Delta smelt and white sturgeon (supported in part by IEP). Both phases will also be coordinated with ongoing efforts by the UCD Aquatic Toxicology Lab (sponsored by CVRWQCB) to assay sites on the Sacramento River and Delta. The proposed study will directly support CALFED's efforts to monitor contaminant effects on native fish and will provide a means of accurately assessing remediation efforts.

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